BRIEF REPORT

Interactive Effects of *DAOA* (G72) and Catechol-*O*-Methyltransferase on Neurophysiology in Prefrontal Cortex

Devon C. Nixon, Morgan J. Prust, Fabio Sambataro, Hao-Yang Tan, Venkata S. Mattay, Daniel R. Weinberger, and Joseph H. Callicott

Background: Accumulating evidence indicates that genetic polymorphisms of D-amino acid oxidase activator (*DAOA*) (M24; rs1421292; T-allele) and catechol-*O*-methyltransferase (*COMT*) (Val^{158}Met; rs4680) likely enhance susceptibility to schizophrenia. Previously, clinical association between *DAOA* M24 (T-allele) and a functionally inefficient 3-marker *COMT* haplotype (that included *COMT* Val^{158}Met) uncovered epistatic effects on risk for schizophrenia. Therefore, we projected that healthy control subjects with risk genotypes for both *DAOA* M24 (T/T) and *COMT* Val^{158}Met (Val/Val) would produce prefrontal inefficiency, a critical physiological marker of the dorsolateral prefrontal cortex (DLPFC) in schizophrenic patients influenced by both familial and heritable factors.

Methods: With 3T blood oxygen level dependent functional magnetic resonance imaging data, we analyzed in SPM5 the proposed interaction of *DAOA* and *COMT* in 82 healthy volunteers performing an *N*-back executive working memory paradigm (2-back > 0-back).

Results: As predicted, we detected a functional gene × gene interaction between *DAOA* and *COMT* in the DLPFC.

Conclusions: The neuroimaging findings here of inefficient information processing in the prefrontal cortex seem to echo prior statistical epistasis between risk alleles for *DAOA* and *COMT*, albeit within a small sample. These in vivo results suggest that deleterious genotypes for *DAOA* and *COMT* might contribute to the pathophysiology of schizophrenia, perhaps through combined glutamatergic and dopaminergic dysregulation.

Key Words: Dopamine, efficiency, fMRI, glutamate, prefrontal, working memory

Several lines of evidence implicate D-amino acid oxidase activator (*DAOA*) (formerly known as G72) and catechol-*O*-methyltransferase (*COMT*) as risk factors in the complex genetic architecture of schizophrenia (1). Allelic variations within and around *DAOA* have been associated with increased susceptibility to schizophrenia (2,3) and to bipolar disorder (3), particularly a polymorphism distal to the coding region (M24; rs1421292; T-allele) (2,3) that predicted cognitive impairments (4) and altered cortical activity (4). *DAOA* transcripts also have been found to be upregulated in dorsolateral prefrontal cortex (DLPFC) tissue of patients with schizophrenia (5). Known for modulating prefrontal synaptic dopamine levels (6), *COMT* (Val^{158}Met; rs4680) was the first of now multiple genes tied to information processing efficiency and therefore offers a promising foundation for exploring gene × gene interactions on cortical efficiency, an important physiological signature of the DLPFC in schizophrenia (7). Previously, Nicodemus *et al.* (8) reported an epistatic interaction between *DAOA* M24 (T-allele) and a functionally inefficient 3-marker *COMT* haplotype (that included *COMT* Val^{158}Met) on risk for schizophrenia (odds ratio: 9.10, 95% confidence interval: 1.37–60.47; odds ratio $p = .02$, likelihood ratio test $p = .02$) (8).

Similar to prior examinations of functional epistasis with *COMT*, we speculated that the *DAOA* × *COMT* interaction, at the level of illness risk, reflects a biological relationship related to risk-associated neural functions. Thus, we expected that normal control subjects possessing both risk genotypes for *DAOA* M24 (T/T) and *COMT* Val^{158}Met (Val/Val) would exhibit DLPFC inefficiency (greater activation for a fixed level of performance) while completing a working memory (WM) task that reliably engages the prefrontal cortex and delineates inefficient information processing in schizophrenic patients that is both familial and heritable (9–11).

Methods and Materials

To explore the proposed link between *DAOA* and *COMT*, we collected data from 82 right-handed healthy volunteers of European ancestry screened for psychiatric and neurological illnesses. Subjects performed an *N*-back executive WM paradigm (2-back > 0-back) during whole-brain 3T blood oxygen level dependent functional magnetic resonance imaging (fMRI) scanning (echo time = 30 msec; repetition time = 2 sec; flip angle = 90°; field of view = 24 cm; matrix = 64 × 64; voxel dimensions = 3.75 × 3.75 × 6 mm) (9). Analyzed data met previously described standards for fMRI scan quality, motion correction, and task performance (9,10,12). Genotype groups did not differ for any of these parameters.

Counts for *DAOA* M24 and *COMT* Val^{158}Met were as follows: *DAOA* (19 A/A; 43 A/T; 20 T/T individuals) and *COMT* (24 Val/Val; 45 Val/Met; 13 Met/Met individuals). Due to cell size limitations for certain *DAOA* × *COMT* combinations, we pooled nonrisk allele carriers for *DAOA* (A/A + A/T) and *COMT* (Val/Met + Met/Met), generating four genotype groups for subsequent epistasis analysis: T/T-Val/Val ($n = 9$), T/T-Met-Carriers ($n = 11$), A-Carriers-Val/Val ($n = 15$), and A-Carriers-Met-Carriers ($n = 47$). Importantly, there was no correlation of genotypes between *DAOA* M24 and *COMT* Val^{158}Met ($r^2 = -.03$, p = ns). Neither *DAOA* M24 [$\chi^2(1) = .20$, p = ns] nor

From the Unit on Dynamic Imaging Genetics (DCN, MJP, H-YT, JHC); Neuroimaging Core Facility (FS, VSM); Genes, Cognition and Psychosis Program (DCN, MJP, FS, H-YT, VSM, DRW, JHC), Clinical Brain Disorders Branch, Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland.

Address correspondence to Joseph H. Callicott, M.D., National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, 10 Center Drive, Room 3C-117, MSC 1379, Bethesda, Maryland 20892-1379; E-mail: callicottj@mail.nih.gov.

Received Mar 26, 2010; revised Oct 28, 2010; accepted Oct 29, 2010.

0006-3223/$36.00
doi:10.1016/j.biopsych.2010.10.031

Table 1. Demographic Data and Behavioral Performance According to *DAOA* × *COMT* Genotype Groups

Genotype Groups[a]	*n*	Age (± SD)	Gender (M:F)[b]	Education (± SD)	Handedness (± SD)	WAIS-IQ (± SD)	2-Back Accuracy (%) (± SD)	2-Back Reaction Times (± SD)
DAOA T/T + *COMT* Val/Val	9	30.4 (10.0)	4:5	16.8 (2.7)	91.1 (9.3)	108 (11.6)	79.8 (21.8)	.585 (.155)
DAOA A-Carriers + *COMT* Val/Val	15	34.2 (7.9)	8:7	16.8 (2.4)	96.0 (8.3)	109 (10.3)	75.6 (17.5)	.573 (.269)
DAOA T/T + *COMT* Met-Carriers	11	33.5 (9.5)	10:1	17.1 (4.6)	93.6 (9.2)	106 (8.0)	87.7 (14.0)	.466 (.173)
DAOA A-Carriers + *COMT* Met-Carriers	47	33.6 (9.6)	23:24	16.8 (2.5)	93.2 (10.0)	106 (8.4)	80.4 (16.9)	.481 (.200)

DAOA, D-amino acid oxidase activator; *COMT*, catechol-*O*-methyltransferase; WAIS, Wechsler Adult Intelligence Scale.
[a]Genotype groups did not significantly differ ($p > .1$, analysis of variance) for any of the study variables.
[b]Statistical trend ($p = .066$, Fisher's exact test).

COMT Val^{158}Met [$\chi^2(1) = 1.14$, p = ns] deviated from Hardy–Weinberg equilibrium.

There were no significant main-effects or interactions ($p < .1$) for age, education, handedness, IQ, and task-related variables (accuracy and reaction time) across genotype groups with analysis of variance (SPSS Version 15.0; SPSS, Chicago, Illinois) (Table 1). Table 1 shows an imbalance in gender distribution across genotypes, with too few female *DAOA* T/T + *COMT* Met-Carriers. We detected a trend for gender differences across genotypes as a result ($p = .066$, Fisher's exact test). We adopted a conservative model with gender as a covariate, given this observation plus other evidence supporting sexually dimorphic effects of *COMT* on brain function (13). To investigate the hypothesized gene × gene interaction, we used a full-factorial random-effects model in SPM5 (http://www.fil.ion.ucl.ac.uk/spm) with *DAOA* (T/T; A-Carriers) and *COMT* (Val/Val; Met Carriers) genotypes as factors and gender included as a nuisance covariate. Examining a gene × gene × gender interaction ideally would require a larger study population, so we could only probe the *DAOA* × *COMT* interaction within a male-only sample ($n = 45$), because we lacked a sufficient number of *DAOA* T/T + *COMT* Met-Carriers female subjects ($n = 1$). Given our a priori assumption regarding the combined impact of *DAOA* and *COMT* on DLPFC efficiency (8), we applied a region-of-interest mask (bilateral intersection of Brodmann areas 9 and 46 with middle frontal gyrus; WFU PickAtlas, Wake Forest University, Winston-Salem, North Carolina) for a small volume correction at family-wise error (FWE) $p < .05$ (search region = 642 voxels, 4.3 resels; corrected SPM5 *T*-threshold = 3.72). To illustrate the interaction, we then extracted SPM parameter estimates taken from the peak voxel and then entered these values in an analysis of covariance (ANCOVA) in SPSS with *DAOA* and *COMT* genotypes as factors and gender as a covariate. In general, analyses performed in SPSS on extracted signals cannot be used to identify regions or interactions that were not present in the SPM5 analysis (14). However, because the value of these findings relies on replication, we include the results of these post hoc tests to provide some sense of the magnitude of effects in this somewhat small sample.

Results

As anticipated, we identified functional epistasis between *DAOA* and *COMT* within the DLPFC [(42, 19, 32, Talairach-Tournoux) (SPM T-contrast: $T = 3.80$, $Z = 3.63$, $p < .05$, FWE small-volume corrected) (SPM F-contrast: $F(1,77) = 14.47$, $p < .0001$, whole-brain uncorrected] (Figure 1A). To represent the interaction, we graphed the extracted peak right DLPFC signal in a post hoc ANCOVA within SPSS—illustrating a *DAOA* × *COMT* interaction [$F(1,77) = 11.93$, $p = .001$] as well as a main-effect of *COMT* [$F(1,77) = 5.83$, $p = .018$] (Figure 1B). Additional post hoc testing with Fisher's least significant difference measure isolated significant differences ($p < .05$) between the among groups: *DAOA* T/T + *COMT* Val/Val > *DAOA* A-Carriers + *COMT* Met-Carriers; *DAOA* T/T + *COMT* Val/Val > *DAOA* A-Carriers + *COMT* Val/Val; *DAOA* T/T + *COMT* Val/Val > *DAOA* T/T + *COMT* Met-Carriers; and *DAOA* A-Carriers + *COMT* Met-Carriers > *DAOA* T/T + *COMT* Met-Carriers. Apart from the hypothesized regions within the DLPFC, exploratory whole-brain inspection did not

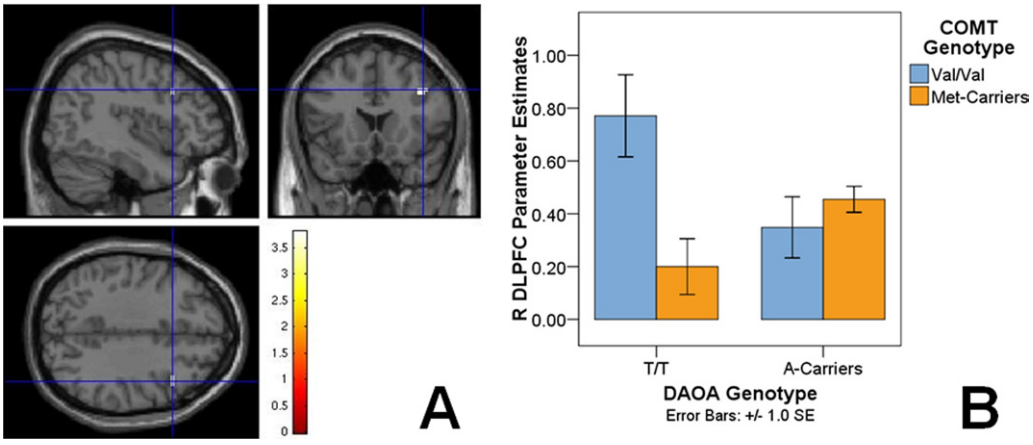

Figure 1. Neuroimaging epistasis between D-amino acid oxidase activator (*DAOA*) and catechol-*O*-methyltransferase (*COMT*) on working memory brain physiology. **(A)** Rendering of the functional epistasis between risk genotypes for *DAOA* M24 (T/T) and *COMT* Val^{158}Met (Val/Val) in the right dorsolateral prefrontal cortex (R DLPFC) (whole-brain display threshold set at $p < .0005$, uncorrected, $k \geq 5$; color gradient represents *T* score). **(B)** When graphed, the extracted functional magnetic resonance imaging signal further reveals the *DAOA* × *COMT* interaction in the DLPFC [SPM F-contrast: $F(1,77) = 14.47$, $p < .0001$; SPSS: $F(1,77) = 11.93$, $p = .001$]. We organized genotypes into the following pairings: *DAOA* T/T + *COMT* Val/Val ($n = 9$), *DAOA* T/T + *COMT* Met-Carriers ($n = 11$), *DAOA* A-Carriers + *COMT* Val/Val ($n = 15$), and *DAOA* A-Carriers + *COMT* Met-Carriers ($n = 47$).

elicit any other areas of supra-threshold activation ($p < .0005$, uncorrected, cluster size (k) ≥ 5).

An analysis of variance in SPM with the full-sample generated results ($T = 3.73, Z = 3.57$ $p < .05$, FWE small-volume corrected; data not displayed) similar to the aforementioned ANCOVA. In just male subjects ($n = 45$), the *DAOA* × *COMT* fMRI interaction produced striking blood oxygen level dependent values at the same DLPFC coordinate as the ANCOVA ($T = 5.54, Z = 4.76, p < .01$, FWE whole-brain corrected; data not displayed).

Discussion

These preliminary findings in a relatively small sample of healthy subjects suggest a functional validation of the statistical epistasis between *DAOA* and *COMT* as demonstrated by Nicodemus *et al.* (8). Putatively, lower synaptic dopamine for *COMT* Val homozygotes (6) coupled with biological effects either directly or via linkage disequilibrium of *DAOA* M24 (T/T homozygotes) act in conjunction to worsen prefrontal dynamics. Analogous disruptions to prefrontal efficiency have been observed in patients with schizophrenia and in their unaffected siblings under identical WM demands (9,10). However, the biological mechanism of the *DAOA* × *COMT* interaction remains uncertain. Early work proposed that *DAOA* modifies *N*-methyl-D-aspartate–receptor function by modulating D-serine levels (2), although subsequent data have raised doubts (15). Interactions between cortical dopamine signaling and *N*-methyl-D-aspartate signaling are well-characterized, and similar interactive effects between *COMT* Val^{158}Met and genetic variation in GRM3, a putative psychosis risk gene related to glutamate synaptic levels, have been reported (16).

These in vivo results also indicate that the neurophysiological impact of *DAOA* and other risk genes like it that fail to produce a significant main-effect of task might emerge only when conditioned on genetic variations, like *COMT*, with well-described effects on a given intermediate phenotype or clinical phenotype like schizophrenia. Although current research estimates that *DAOA* M24 and *COMT* Val^{158}Met each might confer only modest contributions to overall risk for schizophrenia (17), the data here highlight the value of inspecting epistasis between genetic variants of small effect. Epistasis implies nonadditive effects, and this study captured the multiplicative influence of *DAOA* (T/T) and *COMT* (Val/Val) risk genes on prefrontal physiology. The interplay between these genes might also explain the increased activation seen in the *DAOA* A-Carriers + *COMT* Met-Carriers when compared with *DAOA* T/T + *COMT* Met-Carriers and *DAOA* A-Carriers + *COMT* Val/Val. In other words, without *COMT* genotype, *DAOA* effects would be difficult to interpret (18). However, due to the limited number of subjects, particularly the *DAOA* T/T + *COMT* Val/Val cell ($n = 9$), interpretation of these findings should be considered preliminary until replication.

Taken together, our observations encourage further investigation into the biological synergy between *DAOA* and *COMT* on cortical microcircuits. For example, disruption of these circuits might represent a route to and potential therapy for cognitive dysfunction arising from abnormal information processing in the DLPFC of schizophrenic patients, perhaps through combined glutamatergic and dopaminergic pharmacotherapy (1). Absent a conclusive neurobiological mechanism for *DAOA*, these fMRI data intimate that an interaction between *DAOA* and *COMT* risk alleles contributes to the overall pathophysiology of schizophrenia.

This work was graciously supported by the National Institute of Mental Health Intramural Research Program, National Institutes of Health, Bethesda, Maryland. Author FS is currently affiliated with the Brain Center for Motor and Social Cognition, Italian Institute of Technology, Parma, Italy. All authors report no biomedical financial interests or potential conflicts of interest.

1. Harrison PJ, Weinberger DR (2005): Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. *Mol Psychiatry* 10:40–68.
2. Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, *et al.* (2002): Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci U S A* 99:13675–13680.
3. Detera-Wadleigh SD, McMahon FJ (2006): G72/G30 in schizophrenia and bipolar disorder: Review and meta-analysis. *Biol Psychiatry* 60:106–114.
4. Goldberg TE, Straub RE, Callicott JH, Hariri A, Mattay VS, Bigelow L, *et al.* (2006): The, G72/G30 gene complex and cognitive abnormalities in schizophrenia. *Neuropsychopharmacology* 31:2022–2032.
5. Korostishevsky M, Kaganovich M, Cholostoy A, Ashkenazi M, Ratner Y, Dahary D, *et al.* (2004): Is the G72/G30 locus associated with schizophrenia? Single nucleotide polymorphisms, haplotypes, and gene expression analysis. *Biol Psychiatry* 56:169–176.
6. Tunbridge EM, Harrison PJ, Weinberger DR (2006): Catechol-*O*-methyltransferase, cognition, and psychosis: Val158Met and beyond. *Biol Psychiatry* 60:141–151.
7. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, *et al.* (2001): Effect of *COMT* Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 98:6917–6922.
8. Nicodemus KK, Kolachana BS, Vakkalanka R, Straub RE, Giegling I, Egan MF, *et al.* (2007): Evidence for Statistical Epistasis Between Catechol-O-Methyltransferase (*COMT*) and Polymorphisms in RGS4 G72 *DAOA*, GRM3, and DISC1: Influence on risk of schizophrenia. *Hum Genet* 120:889–906.
9. Callicott JH, Mattay VS, Verchinski BA, Marenco S, Egan MF, Weinberger DR (2003): Complexity of prefrontal cortical dysfunction in schizophrenia: More than up or down. *Am J Psychiatry* 160:2209–2215.
10. Callicott JH, Egan MF, Mattay VS, Bertolino A, Bone AD, Verchinksi B, Weinberger DR (2003): Abnormal fMRI response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia. *Am J Psychiatry* 160:709–719.
11. Blokland GA, McMahon KL, Hoffman J, Zhu G, Meredith M, Martin NG, *et al.* (2008): Quantifying the heritability of task-related brain activation and performance during the N-back working memory task: A twin fMRI study. *Biol Psychol* 79:70–79.
12. Callicott JH, Mattay VS, Bertolino A, Finn K, Coppola R, Frank JA, *et al.* (1999): Physiological characteristics of capacity constraints in working memory as revealed by functional MRI. *Cereb Cortex* 1:20–26.
13. Harrison PJ, Tunbridge EM (2008): Catechol-*O*-methyltransferase (*COMT*): A gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. *Neuropsychopharmacology* 33:3037–3045.
14. Kriegeskorte N, Simmons WK, Bellgowan PS, Baker CI (2009): Circular analysis in systems neuroscience: The dangers of double dipping. *Nat Neurosci* 12:535–540.
15. Kvajo M, Dhilla A, Swor DE, Karayiorgou M, Gogos JA (2008): Evidence implicating the candidate schizophrenia/bipolar disorder susceptibility gene G72 in mitochondrial function. *Mol Psychiatry* 13:685–696.
16. Tan HY, Chen Q, Sust S, Buckholtz JW, Meyers JD, Egan MF, *et al.* (2007): Epistasis between catechol-*O*-methyltransferase and type II metabotropic glutamate receptor 3 genes on working memory brain function. *Proc Natl Acad Sci U S A* 104:12536–12541.
17. Allen NC, Bagade S, McQueen MB, Ioannidis JPA, Kavvoura FK, Khoury MJ, *et al.* (2008): Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: The SzGene database. *Nat Genet* 40:827–834.
18. Meyer-Lindenberg NT, Callicott JH, Ding J, Kolachana B, Buckholtz J, Buckholtz J, *et al.* (2006): Impact of complex genetic variation in *COMT* on human brain function. *Mol Psychiatry* 11:867–877.